

### CLAIMS

1           1.    A ligand profile which is characteristic for a  
2   given cell, the ligand profile comprising a representation  
3   of at least ten different polypeptide ligands, all of which  
4   bind to a single type of multi-ligand binding receptor,  
5   wherein the representation characterizes each individual  
6   ligand based upon at least three physical or chemical  
7   attributes; provided that, if the multi-ligand binding  
8   receptor is an MHC class I or class II receptor, at least  
9   500 polypeptide ligands are represented in the ligand  
10  profile; and further provided that the ligand profile is a  
11  reproducible characteristic of the cell.

1           2.    A ligand profile which is characteristic for a  
2   given cell, the ligand profile comprising a representation  
3   of at least ten different polypeptide ligands, all of which  
4   bind to a single type of multi-ligand binding receptor,  
5   wherein the representation characterizes each individual  
6   ligand based upon at least two physical or chemical  
7   attributes, one of said attributes being mass or mass-to-  
8   charge ratio; provided that, if the multi-ligand binding  
9   receptor is an MHC class I or class II receptor, at least  
10  500 polypeptide ligands are represented in the ligand  
11  profile; and further provided that the ligand profile is a  
12  reproducible characteristic of the cell.

1           3.    A ligand profile which is characteristic for a  
2   given cell, the ligand profile comprising a representation  
3   of at least ten different polypeptide ligands, all of which  
4   bind to a single type of multi-ligand binding receptor,  
5   wherein the representation characterizes each individual  
6   ligand based upon at least one physical or chemical  
7   attribute, the at least one physical or chemical attribute

8 comprising amino acid sequence; provided that, if the multi-  
9 ligand binding receptor is an MHC class I or class II  
10 receptor, at least 50 polypeptide ligands are represented in  
11 the ligand profile; and further provided that the ligand  
12 profile is a reproducible characteristic of the cell.

1           4. A ligand profile which is characteristic for a  
2 given cell, the ligand profile comprising ion fragmentation  
3 patterns for at least ten different polypeptide ligands, all  
4 of which polypeptide ligands bind to a single type of multi-  
5 ligand binding receptor; provided that, if the multi-ligand  
6 binding receptor is an MHC class I or class II receptor, at  
7 least 100 polypeptide ligands are represented in the ligand  
8 profile; and further provided that the ligand profile is a  
9 reproducible characteristic of the cell.

1           5. A ligand profile which is characteristic for a  
2 given cell, the ligand profile comprising amino acid  
3 sequences of at least ten different polypeptide ligands  
4 having distinct core peptides, all of which ligands bind to  
5 a single type of multi-ligand binding receptor; provided  
6 that, if the multi-ligand binding receptor is an MHC class I  
7 or class II receptor, at least 100 polypeptide ligands are  
8 represented in the ligand profile; and further provided that  
9 the ligand profile is a reproducible characteristic of the  
10 cell.

1           6. The ligand profile of claim 1, wherein the  
2 multi-ligand binding receptor is an MHC class I or MHC class  
3 II receptor.

1           7.    The ligand profile of claim 1, wherein the  
2 multi-ligand binding receptor is not an MHC class I or MHC  
3 class II receptor.

1           8.    The ligand profile of claim 1, wherein the  
2 multi-ligand binding receptor is a chaperone, a chaperonin,  
3 a calnexin, a calreticulin, a mannosidase, a N-glycanase, a  
4 BIP, a grp94, a grp96, hsp60, hsp65, hsp70, hsp90, hsp25, an  
5 E2 ubiquitin carrier protein, an E3 ubiquitin ligase, an  
6 unfoldase, hsp100, a proteasome, a trafficking protein, or a  
7 retention protein.

1           9.    The ligand profile of claim 1, combined with a  
2 second ligand profile, the second ligand profile (a) also  
3 being a reproducible characteristic of the given cell, and  
4 (b) comprising a representation of at least ten additional  
5 polypeptide ligands, all of which bind to a second type of  
6 multi-ligand binding receptor different from the first type  
7 of receptor.

1           10.   A method of generating a reproducible ligand  
2 profile for a given cell type, which cell type comprises a  
3 selected type of multi-ligand binding receptor, the method  
4 comprising:

5           (a)   providing a first sample of the given cell  
6 type, wherein the first sample comprises a first plurality  
7 of polypeptide ligands bound to the selected type of multi-  
8 ligand binding receptor;

9           (b)   isolating the selected type of multi-ligand  
10 binding receptor from the first sample;

11          (c)   separating the first plurality of ligands from  
12 the selected type of multi-ligand binding receptor;

13          (d)   fractionating the first plurality of ligands;

14           (e) generating a first profile distinguishing among  
15 the first plurality of ligands on the basis of at least one  
16 chemical or physical attribute;  
17           (f) providing a second sample of the given cell  
18 type, the second sample being essentially identical to the  
19 first sample, wherein the second sample comprises a second  
20 plurality of polypeptide ligands bound to the selected type  
21 of multi-ligand binding receptor;  
22           (g) isolating the selected type of multi-ligand  
23 binding receptor from the second sample;  
24           (h) separating the second plurality of ligands from  
25 the selected type of multi-ligand binding receptor;  
26           (i) fractionating the second plurality of ligands;  
27           (j) generating a second profile distinguishing  
28 among the second plurality of ligands on the basis of the at  
29 least one chemical or physical attribute; and  
30           (k) confirming that the first profile and the  
31 second profile are essentially identical, and together  
32 represent a reproducible ligand profile for the given cell  
33 type.

1           11. The method of claim 10, wherein a second  
2 chemical or physical attribute of each ligand is determined  
3 subsequent to the fractionation steps, and is represented in  
4 the profiles.

1           12. The method of claim 11, wherein a third  
2 chemical or physical attribute of each ligand is determined  
3 subsequent to the fractionation steps, and is represented in  
4 the profiles.

1           13. The method of claim 10, wherein the isolating  
2 and separating steps are accomplished using appropriate  
3 columns arranged in an in-line system.

1           14. A method of generating a ligand profile for a  
2 given type of cell, comprising:

3           (a) providing a sample of lysate of the given type  
4 of cell, wherein the sample comprises a first plurality of  
5 polypeptide ligands bound to a first type of multi-ligand  
6 binding receptor and a second plurality of polypeptide  
7 ligands bound to a second type of multi-ligand binding  
8 receptor;

9           (b) isolating the first and second types of multi-  
10 ligand binding receptors from the sample;

11           (c) separating the first plurality of ligands from  
12 the first type of multi-ligand binding receptor and the  
13 second plurality of ligands from the second type of multi-  
14 ligand binding receptor;

15           (d) fractionating the first plurality of ligands  
16 and the second plurality of ligands; and

17           (e) generating a first profile distinguishing among  
18 the first plurality of ligands on the basis of at least one  
19 chemical or physical attribute and a second profile  
20 distinguishing among the second plurality of ligands on the  
21 basis of the same at least one chemical or physical  
22 attribute.

1           15. A method of generating a subtraction profile of  
2 polypeptide ligands, comprising:

3           (a) producing a first ligand profile by a method  
4 comprising:

5           (i) providing a first sample comprising a  
6 first cell of interest, wherein the first cell of interest

7 comprises a given type of multi-ligand binding receptor  
8 bound to a first set of polypeptide ligands;  
9 (ii) isolating the given type of multi-ligand  
10 binding receptor and the first set of ligands from the first  
11 sample;  
12 (iii) separating the first set of ligands from  
13 the given type of multi-ligand binding receptor;  
14 (iv) generating a first profile distinguishing  
15 among the first set of ligands on the basis of at least one  
16 chemical or physical attribute;  
17 (b) producing a second profile of ligands by a  
18 method comprising:  
19 (i) providing a second sample comprising a  
20 second cell of interest, wherein the second cell of interest  
21 comprises the given type of multi-ligand binding receptor,  
22 bound to a second set of polypeptide ligands;  
23 (ii) isolating the given type of multi-ligand  
24 binding receptor and the second set of ligands from the  
25 second sample;  
26 (iii) separating the second set of ligands from  
27 the given type of multi-ligand binding receptor;  
28 (iv) generating a second profile  
29 distinguishing among the second set of ligands on the basis  
30 of the same at least one chemical or physical attribute;  
31 (c) comparing the first profile and the second  
32 profile to identify differentially expressed ligands,  
33 thereby forming a subtraction profile of ligands.

1 16. A subtraction profile generated by the method  
2 of claim 15.

1 17. A method of comparing a first cell sample to a  
2 reference cell sample, comprising:

3           (a) producing a first ligand profile by a method  
4 comprising:  
5           (i) providing a first cell sample comprising  
6 a given type of multi-ligand binding receptor bound to a  
7 first set of polypeptide ligands;  
8           (ii) isolating the given type of multi-ligand  
9 binding receptor and the first set of ligands from the first  
10 cell sample;  
11           (iii) separating the first set of ligands from  
12 the given type of multi-ligand binding receptor;  
13           (iv) generating a first ligand profile  
14 distinguishing among the first set of ligands on the basis  
15 of at least one chemical or physical attribute;  
16       (b) providing a reference ligand profile  
17 representing a second set of polypeptide ligands extracted  
18 from the given type of multi-ligand binding receptor of a  
19 reference cell sample, wherein the reference ligand profile  
20 distinguishes among the second set of polypeptide ligands on  
21 the basis of the at least one chemical or physical  
22 attribute; and  
23       (c) comparing the first ligand profile to the  
24 reference ligand profile, in order to identify differences  
25 or similarities between the first cell sample and the  
26 reference cell sample.

1           18. The method of claim 17, wherein the reference  
2 cell sample consists essentially of healthy cells of an  
3 animal and the first cell sample comprises cells suspected  
4 of being diseased.

1           19. The method of claim 17, wherein the first cell  
2 sample comprises cells cultured in the presence of a test  
3 compound, and the reference cell sample does not.

1           20. The method of claim 17, wherein the reference  
2 cell sample comprises cells cultured in the presence of a  
3 test compound, and the first cell sample does not.

1           21. A set of ligand profiles, comprising

2           (a) a first ligand profile comprising a first  
3 representation of a first plurality of polypeptide ligands,  
4 all of which bind to at least one multi-ligand binding  
5 receptor of a first cell, wherein the first representation  
6 distinguishes among the members of the first plurality of  
7 ligands based upon at least one physical or chemical  
8 attribute; and

9           (b) a second ligand profile comprising a second  
10 representation of a second plurality of polypeptide ligands,  
11 all of which bind to the at least one type of multi-ligand  
12 binding receptor of a second cell, wherein the second  
13 representation distinguishes among the second plurality of  
14 ligands based upon the at least one physical or chemical  
15 attribute;  
16 provided that (i) the first cell differs from the second  
17 cell in a parameter selected from the group consisting of  
18 genetic background, culture conditions, genetic background  
19 plus culture conditions, *in vivo* exposure to a test  
20 compound, and genetic background plus *in vivo* exposure to a  
21 test compound; and (ii) any significant difference between  
22 the first and the second ligand profiles is attributable to  
23 that parameter.

1           22. A method of detecting a difference between the  
2 set of proteins expressed in a first cell and the set of  
3 proteins expressed in a second cell, comprising

4           (a) providing a first ligand profile made by a  
5 method comprising



6                   (i)     providing a first cell comprising at  
7     least one type of multi-ligand binding receptor, bound to a  
8     first set of polypeptide ligands,  
9                   (ii)    isolating from the first cell the at least  
10    one type of multi-ligand binding receptor bound to the first  
11    set of ligands,  
12                  (iii)   separating the first set of ligands from  
13    the at least one type of multi-ligand binding receptor, and  
14                  (iv)    generating a first ligand profile  
15    distinguishing among the members of the first set of ligands  
16    on the basis of at least one chemical or physical attribute;  
17       (b)   providing a second ligand profile made by a  
18    method comprising  
19                  (i)     providing a second cell comprising the at  
20    least one type of multi-ligand binding receptor, bound to a  
21    second set of polypeptide ligands,  
22                  (ii)    isolating from the second cell the at  
23    least one type of multi-ligand binding receptor, bound to  
24    the second set of ligands,  
25                  (iii)   separating the second set of ligands  
26    from the at least one type of multi-ligand binding receptor,  
27    and  
28                  (iv)    generating a second ligand profile  
29    distinguishing among the members of the second set of  
30    ligands on the basis of the at least one chemical or  
31    physical attribute;  
32       (c)   comparing the first ligand profile to the  
33    second ligand profile, in order to identify any difference  
34    between the first and second profiles, wherein such a  
35    difference is an indication of a difference between the set  
36    of proteins expressed in the first cell and the set of  
37    proteins expressed in the second cell.

1           23. The method of claim 22, comprising the further  
2 step of

3           (d) generating a differential profile which sets  
4 forth at least some of the differences between the set of  
5 proteins expressed in the first cell and the set of proteins  
6 expressed in the second cell.

1           24. A differential profile generated by the method  
2 of claim 23.

1           25. The method of claim 22, comprising the further  
2 steps of selecting a ligand which is represented in one  
3 profile but not in the other, and identifying the amino acid  
4 sequence of the ligand.

1           26. A database, stored on a machine-readable  
2 medium, comprising  
3           three categories of data respectively representing  
4 (a) ligand profiles, (b) cell sources, and (c) receptor  
5 types, and  
6           associations among instances of the three categories  
7 of data,  
8           wherein the database configures a computer to enable  
9 finding instances of data of one of the categories based on  
10 their associations with instances of data of another one of  
11 the categories.

1           27. The database of claim 26 in which data  
2 representing the cell sources comprise data identifying at  
3 least one type of cell.

1           28. The database of claim 26 in which data  
2 representing the cell sources comprise data identifying at  
3 least one cell condition.

1           29. The database of claim 26 in which data  
2 representing the cell sources comprise data identifying at  
3 least one individual animal.

1           30. The database of claim 26 in which data  
2 representing the cell sources comprise data identifying at  
3 least one state of perturbation.

1           31. The database of claim 26 in which data  
2 representing the cell sources comprise data identifying at  
3 least one developmental state.

1           32. The database of claim 26 in which the ligand  
2 profiles comprise information that uniquely identifies  
3 protein fragments.

1           33. The database of claim 26 in which the ligand  
2 profiles comprise mass spectral data.

1           34. The database of claim 26 in which the database  
2 configures the computer to enable finding at least one  
3 instance of the ligand profiles that is associated with a  
4 selected one or more instances of the cell sources and a  
5 selected one or more instances of the receptor types.

1           35. A machine-implemented method comprising  
2 forming a query for searching a database, the  
3 database comprising three categories of data respectively  
4 representing (a) ligand profiles, (b) cell sources, and (c)

5 receptor types, the database defining associations among  
6 instances of the three categories of data, the query  
7 comprising one or more instances of one of the three  
8 categories of data, and  
9 applying the query to the database to find instances  
10 of another one of the three categories of data.

1 36. The method of claim 35 in which the found  
2 instances comprise two ligand profiles.

1 37. The method of claim 36 further comprising  
2 comparing the two ligand profiles to determine a  
3 difference between them.

1 38. The method of claim 36 in which the query  
2 comprises instances of a selected cell source comprising a  
3 selected cell condition.

1 39. A machine-based method comprising  
2 performing an experiment on cells,  
3 identifying a ligand profile associated with said  
4 cells, and  
5 based on the ligand profile, querying a database  
6 that contains at least two categories of data, including  
7 ligand profiles and cell sources, to derive a cell source or  
8 a ligand profile and an associated cell source.

1 40. The method of claim 39 in which  
2 the feature of the experiment comprises treatment of  
3 the cells using a candidate drug regimen, and  
4 a cell source identified as a result of the query  
5 represents a different treatment of cells.

1           41. A machine-assisted method of investigation  
2 comprising  
3           identifying a cell source, a receptor type, or a  
4 ligand profile of interest, and  
5           based on the identified cell source, receptor type,  
6 or ligand profile, querying a database that contains three  
7 associated categories of data respectively representing (a)  
8 ligand profiles, (b) cell sources, and (c) receptor types,  
9 to derive information about cell sources, receptor types, or  
10 ligand profiles that relates to the cell source, receptor  
11 type, or ligand profile of interest.

1           42. A machine-assisted method comprising  
2 providing cells of a cell source,  
3 generating a ligand profile from the cells, and  
4 based on the ligand profile and the cell source,  
5 querying a database that contains three associated  
6 categories of data respectively representing (a) ligand  
7 profiles, (b) cell sources, and (c) receptor types, to  
8 derive information about cell sources, receptor types, or  
9 ligand profiles that relates to the provided cell source and  
10 the generated ligand profile.